Hemolysin Formation in Mice Following Partial, Total Splenectomy or Spleen Transplantation. (24940)

FALCONER SMITH, MARIE M. GRENAN AND KRISTIN LUNDE
National Cancer Inst., Dept. HEW, Bethesda, Md.

Importance of the spleen in fixation of antigens(1) and in regulation of antibody formation(2,3) has long been recognized. Current interest in immunologic problems encountered in foreign tissue transplantation experiments, especially in radiation protection investigations(4,5), stimulates the search for methods of reducing unfavorable host reactions. The present study was undertaken to determine the contribution of spleen to primary hemolysin response of mice.

Methods. Surgical removal of part or all of the spleen was done within 24 hours of birth in mice of both sexes of inbred NIH strain. Other groups of male mice of same strain were similarly partially splenectomized at 11 weeks of age. Sham surgical operations were performed on littermate animals of both ages for control purposes. Veterinary nembutal (0.3 mg/g body weight) was used as anaesthetic for older animals and ether for infant mice. In transplantation studies, described in detail later, splenectomized mice of 2 unrelated strains, NIH and Balb/c, served both as donors and recipients of whole spleens, each cut into 4 pieces. Splenectomy was immediately followed by transplantation of tissue into peritoneal cavity of recipients and immunization was done 21 days later. Mice were inoculated intravenously or intraperitoneally at 12 to 15 weeks of age with 2.5% suspension of sheep erythrocytes given as 0.01 ml/g of body weight. Serum was collected at sacrifice 5 days after immunization and the serum hemolysin content was estimated colorimetrically as described by Taliaferro(6). Spleens from control mice, spleen tissue fragments and remnants of spleens of partially splenectomized mice were weighed after blotting at time of bleeding.

Results. From the trend indicated by distribution of individual serum hemolysin titers shown in Fig. 1 it is concluded that the capacity of mice to form antibody was interfered with when the amount of remnant spleen was reduced below .2 to .3% of body weight. Average spleen weight for 11 mice (avg. wt. = 28.9 g) given sham splenectomy as adults was 154 $\pm$ 13 mg (S.E.) and for 25 mice (avg. wt. = 25.8 g) similarly treated within 24 hours of birth was 122 $\pm$ 3 mg. Corresponding average hemolysin titers were $-2.6 \pm .03$ and $-2.7 \pm .1$ respectively. Removal on the average of more than 45 to 55% of spleen tissue in mice was associated with reduced capacity for hemolysin formation.

Total splenectomy in NIH mice completely suppressed hemolysin formation when the antigen was given intravenously 21 days after surgery. Results of independent experiments demonstrated that average hemolysin titers of groups of mice of 5-10 animals/group, bled 6 days after immunization on day of splenectomy or 7 or 14 days thereafter, did not differ significantly from average titers observed in mice immunized at 21 days nor was there any detectable change in time required for development of peak titer. A reduction both in rate and amount of antibody formed was observed following intraperitoneal immunization of splenectomized mice because the average hemolysin titer in 9 intact mice on 5th day after antigen inoculation was $-2.68 \pm .1$ (S.E.) compared to only $-1.97 \pm .1$ for 10 splenectomized mice 6 days after immunization. It is of interest to note that in other experiments increases to 30% over average control values in the amount of spleen tissue by transplantation of isologous spleen to groups of otherwise intact Balb/c mice were ineffective in increasing their average hemolysin titers.

Since transplanted pieces of spleen did not increase hemolysin titers of mice, observations were made on the effect of transplanted spleens of autologous, isologous or homologous origin on hemolysin formation of splenectomized recipients. Table I gives average se-

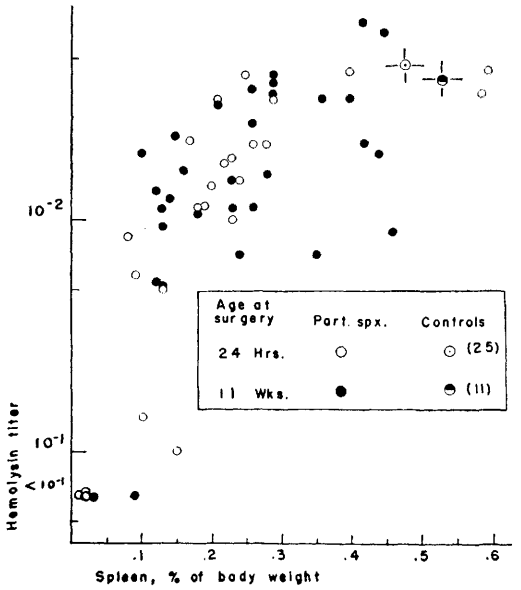

FIG. 1. Individual serum hemolysin titers of mice with the indicated amounts of spleen tissue compared to the averages observed in corresponding sham-operated controls. Numbers in parentheses indicate numbers of control mice/group and bars represent 95% confidence limits of the means.

rum hemolysin titer 5 days after a single intravenous immunizing injection made 21 days after splenectomy and spleen transplant in 2 strains of mice. When the mouse spleen was removed, cut into 4 pieces and returned to peritoneal cavity of same animal, the 5th day

TABLE I. Hemolysin Formation in 2 Strains of Splenectomized Mice Receiving Whole Spleen Transplants of Autologous (A),* Isologous (I)* or Homologous Origin.

Strain		No.	Avg hemolysin† titer
Donor	Host	mice	± S.E.
NIH controls		15	$-2.77 \pm .04$
Balb/c controls		10	$-2.91 \pm .05$
NIH	NIH(A)	25	$-1.24 \pm .19$
Balb/c	Balb/c(A)	13	$-1.87 \pm .09$
NIH	NIH(I)	18	16 = <-1.0; $-1.25, -2.45$
Balb/c	Balb/c(I)	13	$-1.49 \pm .14$
NIH	Balb/c	10	<-1.
Balb/c	NIH	10	"
NIH	NIH(A)‡	5	"

* Spleen was surgically removed, cut into 4 pieces and returned to the peritoneal cavity of same animal (A) or another animal (I).

† Serum hemolysin was estimated at 5 days after intrav. immunization 21 days after splenectomy and transplant.

‡ Each spleen was removed, macerated in Potter type homogenizer in Tyrode's solution and resulting cell suspension intrav. injected.

titers averaged –1.24 and –1.87 for 25 NIH and 13 Balb/c mice respectively. Average titers were significantly lower when isologous transplants were made in both strains as shown in Table I and homologous transplants were associated with a complete suppression of the hemolysin response. A few NIH mice which received their own spleens as suspensions of cells were unable to form hemolysin after intravenous immunization. Gross observation of the condition of transplanted pieces of spleen supplied evidence that in general good "takes" with apparently rich vascularization were associated with antibody formation. However, isologous transplants in 7 of the 18 NIH mice were judged as good "takes" at autopsy, but only 2 of these mice formed detectable amounts of hemolytic antibody. All but one of the 13 Balb/c mice which received isologous spleens had healthy appearing transplants and this animal failed to produce hemolysin on challenge. This discrepancy between the 2 strains may be attributed to genetic differences arising from breeding procedures.

From the observation that antibody titers averaged only about 27% less and rate of production was somewhat reduced in splenectomized mice given antigen by intraperitoneal route, it is suggested that spleens of intact mice served chiefly as filter organs for the particulate antigen. This possibility was substantiated by the observation that transplanted autologous spleen cell suspensions did not improve hemolysin titers of splenectomized NIH mice while at least some antibody was formed in mice with transplanted splenic tissue characterized by good "takes."

Summary. Serum hemolysin titers were correlated with weight of remnant spleen present in groups of partially splenectomized NIH mice sacrificed 5 days after intravenous immunization with sheep erythrocytes. Total splenectomy of mice completely suppressed hemolysin formation after intravenous immunization and was associated with a 27% decrease in amount of antibody formed, and a 24 hour delay required for development of peak serum titer. Complete suppression of antibody formation was partially reversed by

autologous spleen transplants in splenectomized mice of 2 strains; by isologous transplants in Balb/c but not in NIH mice; and unaffected by homologous transplants in either of the 2 strains.

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Neonatal Bilirubin Levels Following Administration of l-Triiodothyronine.* (24941)

MILTON WESTPHAL, THOMAS R. BOGGS, JR., WALTER R. EBERLEIN AND ALFRED M. BONGIOVANNI (Introduced by T. N. Harris)

Children's Hospital of Philadelphia, Pa. Hospital and University of Pennsylvania

Association of prolonged jaundice with cretinism was noted by Christensen(1). He suggests that lack of thyroid hormone may delay hepatic enzyme maturation just as it delays maturation of ossification centers. When Christensen gave thyroid hormone to cretinous infants, there was a prompt fall in their serum bilirubin. His observations prompted us to undertake a study of the effect of thyroid substance on physiologic hyperbilirubinemia of the newborn. l-Triiodothyronine† was selected for administration to infants because of rapidity of its action.

Methods. Using the system of alternates, 100 test and 100 control infants were selected upon admission to nursery. All infants were normal, full term and were admitted to nursery from delivery. Each test infant was given oral dose of 25 μg of l-triiodothyronine daily for 4 days. Control infants were given no medication. Bilirubin determinations were done on each infant at 24-48 hours and 72-96 hours of age. Blood samples were drawn during morning hours by heel puncture technic. Bilirubin was measured by method of Hsia (2) as modified by C. C. Roby (personal communication). Levels of direct bilirubin were low, hence only total bilirubin was considered in calculations. The effect of l-triiodothyronine on bilirubin disposition was evaluated by comparing mean difference between first and second bilirubin levels in test and control groups, by comparing means of 24-48 and 72-96 hour specimens in test group with corresponding means in control group, and by comparing number of infants in each group who showed a rise in serum bilirubin during interval between the 2 determinations. Red cells of all infants were tested by direct Coombs technic for presence of blocking antibodies. ABO and Rh typings were done on all infants and their mothers.

Results. The mean difference between first and second bilirubin determinations in babies to whom l-triiodothyronine was given, was $+ 0.53 \pm 0.28$ mg/100 ml. The mean difference between the 2 specimens in control group was $+ 1.6 \pm 0.31$ mg/100 ml ($t = 7.0$; $p<0.01$). There was no evidence that the 2 groups differed in variability. Mean bilirubin level in test infants at 24-48 hours was 5.0 ± 0.25 mg/100 ml, range 0.8-12.2 mg/100 ml and at 72-96 hours was 5.5 ± 0.41 mg/100 ml, range 0.0-16.5 mg/100 ml. In control infants, the mean for first specimen was 5.8 ± 0.26 mg/100 ml (range, 0.8-13.4 mg/100 ml) and for second specimen, 7.3 ± 0.45 mg/100 ml (range, 0.6-19.3 mg/100 ml)

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